Antioxidant Effect of a Mixture of Three Plants Traditionally used against Diabetes

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Abstract

Medicinal plants are often used as a mixture to benefit from their complementary or synergistic effect. The objective of our study is to evaluate in vivo hypoglycemic activity of a mixture of three medicinal plants Ajuga iva, Peganum harmala and Salvia officinalis associated with reactive oxygen species in the liver of rats made diabetic by streptozotocin. The work is initiated by a preparation of the aqueous extracts from the dried plant material, followed by a phytochemical study of the extracts obtained. Male rats are made diabetic by injection of a single dose of 60 mg/kg of streptozotocin. At the end of the experiment, the liver is removed and a cytosolic assay of catalase, superoxide dismutase, glutathione and malondialdehyde (MDA) is performed to estimate possible oxidative damage caused by the onset of diabetes.The phytochemical tests carried out reveal the presence of the different families of chemical compounds existing at different levels depending on the plant studied . Treatment of the diabetic rats with the mixture extract caused a significant decrease ($p < 0.05$) in the serum glucose concentration, causing a significant improvement in antioxidant status in the liver by reducing the concentration of MDA and increased glutathione levels, the CAT activity and that of SOD. Our results reveal that the metabolites contained in the mixture produced hypoglycemic, antioxidant and cytoprotective effects by reducing the production of MDA and normalizing the cytosolic level of antioxidant systems, which helps to reduce the development of complications associated with diabetes.

Keywords: *Ajuga iva, Peganum harmala, Salvia officinalis, hypoglycemia ,oxidative stress*

INTRODUCTION

Diabetes is a chronic disease resulting from a metabolic disorder, characterized by a rise in blood glucose above values that can lead to specific tissue complications [1] . This pathology is a major and growing problem in a public health , affecting almost 10% of the world's population [2].

Patients with diabetes have high oxidative stress and impaired antioxidant defense systems, which contribute to the initiation and progression of diabetic-induced complications [3] . The existing antioxidant defense systems in the body are likely to modulate the level of intracellular oxidative stress. Indeed, superoxide dismutase, catalase and glutathione peroxidase can catalyze the dismutation of free radicals or their neutralization [4]. Malondialdehyde reflects the degree of lipid peroxidation, the increase in its production plays an important role in controlling the progression of diabetes [5]. Despite the use of hypoglycemic agents as antidiabetic drugs, diabetes and its complications are a major problem in the treatment of diabetic patients. Regular administration of modern medications, including insulin and oral hypoglycemic agents (biguanides, sulfonylureas), produces side effects [6]. Recently, diabetologists have come to the conclusion that a therapeutic supplement consisting of plant extracts is necessary to optimize the treatment of diabetes [7] .

Plants are recognized as a wonderful source of pharmaceuticals. Currently 1200 plant species are used as drugs in the traditional therapy of diabetes. However, for most of them, the scientific evidence is still unclear. Indeed, human life on earth is closely linked to the exploitation of plants. Their beneficial effect is attributed to their antioxidant powers, mediators of the inflammatory response, antiviral, hepatoprotective, cardiovascular, antidiabetic, cancer, Alzheimer's, parkinson's [8].

Among these plants used in traditional medicine *Ajuga iva* L. (ivette) widely distributed in the arid regions of Europe, Asia, Africa and Australia, In is used to treat diabetes and hypertension [9] . *Peganum harmala* (Harmel) plant growing in the arid and semi-arid areas of North Africa and the Middle East, is widely used in Algeria to treat diabetes.

Salvia officinalis Euro-Mediterranean species, is cited in several ethnobotanical and ethno-pharmacological surveys for the treatment of diabetes, as emmenagogue, cholagogue, diuretic, antiseptic, anti-inflammatory, stimulant, choleretic, antispasmodic, hypoglycemic, astringent, warming [10].

This work aims to study the evaluation of the antidiabetic and antioxidant activity of the lyophilized aqueous extract of a mixture of three medicinal plants of *Peganum harmala*, *Ajuga iva* and *Salvia officinalis* in healthy rats and made diabetic by Straptozotocin.

MATERIALS AND METHODS

Preparation of plant material

Ajuga iva, *Peganum harmala*, *Salvia officinalis*, were collected in February 2016 in the region of Bouira (Est of Algiers, Algeria).The leaves of the plant are dried, crushed and stored in glass vials protected from light and moisture for subsequent analysis.

Phytochemical characterization

The phytochemical tests carried out on the three plants *Ajuga iva*, *Peganum harmala* and *Salvia officinalis*, aim to find the bioactive substances. They are made either on the powder or on the infused at 10%. The characterization methods used are from those described by [11].

Extraction and dosage of polyphenols

The aqueous extract of the three plants is obtained from 100 g of dried and ground leaves and then refluxed in distilled water for one hour and finally filtered. The filtrate is centrifuged for 30 minutes at 5000 rpm. The supernatant was filtered to remove any residues. The filtrate was lyophilized until a brown powder was obtained. The extract of the mixture is obtained by grinding 10 g of each plant [12].

The total phenol content of the extracts was determined by the method of Folin-Ciocalteu, mixture of phosphotungstic acid complexes ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) of yellow color, described by Wong [13]. The principle of the method is based on the oxidation of phenolic compounds by this reagent, resulting in the formation of a new blue-colored molybdenum-tungsten complex that absorbs at 760 nm. The test in question consists in mixing a quantity of 0.5 ml of the extract at different concentrations with 1 ml of the freshly prepared Folin-Ciocalteu reagent (10 times diluted) and 1 ml of 20% sodium carbonate (Na_2CO_3). The whole is incubated in the dark and at room temperature for 60 minutes and the reading is performed against a blank using a spectrophotometer at 760 nm. The quantification of the polyphenols was determined according to a calibration curve, made by gallic acid at

different concentrations, under the same conditions as the sample. The results are expressed in milligrams equivalent of gallic acid per gram of dry vegetable matter.

In vitro evaluation of antioxidant activity

The evaluation of the antioxidant effect is carried out by the DPPH free radical scavenging test, using the method described by Sánchez-Moreno [14]. This study is based on scavenging the stable free radical DPPH by an anti-radical molecule, which causes discoloration. The method is rapid and convenient to implement. It is carried out at ambient temperature, allowing eliminating any risk of thermal degradation of the molecules tested. For this, a volume of 25 µl of each methanolic extracts at various concentrations (0.0125 to 1 mg/ml) is added to 1.95 ml of the methanolic solution of DPPH (0.024 g/l). In parallel, a negative control is prepared by mixing of methanol with 1.975 ml of the methanolic solution of DPPH. The reading of the absorbance is made against a blank prepared for each concentration at 517 nm after 30 min of incubation in the dark and at room temperature. The positive control is represented by a solution of a standard antioxidant, ascorbic acid, whose absorbance is measured under the same conditions as the samples. The test is repeated three times for each concentration. The results are expressed as percent inhibition (I %) according to the following formula:

$$I\% = \frac{(\text{Abs control} - \text{Abs test})}{\text{Abs control}} \times 100$$

The IC_{50} values are determined graphically by linear regression

In vivo evaluation of antioxidant activity

The antioxidant power of the mixture is evaluated in vivo on two groups of 5 male Wistar rats of average weight of 120 g each, respectively pretreated orally with a dose of 300 mg/kg of polypenols and gallic acid, before and after two hours of intraperitoneal injection of streptozotocin at a dose of 40 mg/kg body weight according to the modified experimental protocol of Kebiéche [15]. The third group receives a physiological solution in addition to the injection of streptozotocin. The fourth group is considered as a control.

After treatment, the rats were killed and the livers were taken out in order to measure the activity of liver antioxidant enzymes.

The animals were cared for in accordance with the criteria outlined in the “Guide for the Care and Use of Experimental Animals” prepared by the National Academy of Sciences and published by the National Institute of Health.

Estimation of lipid peroxidation

The method described by Ohkawa [16] was used to assess lipid peroxidation in the liver by assaying MDA, one of the end products of the decomposition of polyunsaturated fatty acids during stress as a result of free radicals released. For this, 1 g of liver supplemented with 3 ml of KCl solution (1.15 M) is ground by a homogenizer. 0.5 ml of trichloroacetic acid at 20% and 1 ml of thiobarbituric acid (TBA) to 0.67% are added to 0.5 ml of the homogenate. The mixture is heated at 100 °C for 15 minutes. Once cooled, 4 ml of butanol are added. After centrifugation for 15 minutes at 3000 rpm, the absorbance is determined on the supernatant using a spectrophotometer at 532 nm. The concentration of MDA is deducted from a standard range established in the same conditions with 1,1,3,3 tetraetoxypropane which gives the MDA after its hydrolysis in solution. The assay results are expressed in nmol/g liver.

Hepatic glutathione assay (GSH)

The colorimetric method of Ellman [17] was used for assaying GSH. One gram of liver was homogenized in three volumes of trichloroacetic acid (TCA) 5% using a crusher and then centrifuged at 2000 rpm for 15 minutes. Fifty microliters of supernatant are diluted in 10 ml of phosphate buffer (0.1 M; pH 8). 20 µl of 55 'dithiodis-2-nitrobenzoic DTNB (0.01 M) are added to 3 ml of the dilution mixture. The absorbance is read at 412 nm against a blank prepared in the same conditions with 5% of TCA. The concentrations are deducted from a standard range established under the same conditions with the glutathione. They are expressed in nmol/g liver.

Dosage of the enzymatic activity of catalase

For this test, a cytosolic fraction is prepared by the method of Iqbal [18], starting from 2 g of liver coupes and homogenized in three volumes of phosphate buffer (0.1 M; pH 7.4) containing KCl (1.17%) by a homogenizer. The homogenate is centrifuged at 2000 rpm for 15 minutes at 4 °C. The supernatant obtained is centrifuged at 9600 rpm for 30 minutes at 4 °C, and the final supernatant represents the fraction used for the evaluation of the activity of enzymes (CAT and SOD). The evaluation of the enzymatic activity of CAT was determined by the method of Claiborne [19]. For this, the rate constant of the reaction is determined from a mixture consisting of 1 ml of phosphate buffer (0.1 M; pH 7.2), 0.975 ml of freshly prepared H2O2 (0.091 M) and 0.025 ml of the cytosolic

fraction, after reading the absorbance at 560 nm every minute for two minutes according to the following formula:

$$K = \frac{2.303}{T} \times \frac{logA1}{A2}$$

K: Constant

T: Time interval

A_1: Absorbance in zero time

A_2: Absorbance after a minute

The enzymatic activity is calculated in terms of international units per minute and per gram of protein (U/min per gram of protein) using the following equation:

$$UI/mg\ protetnes = \frac{K}{N}$$

N: amount of protein in mg present in the volume of the sample used.

IU/mg protein: µmol of H_2O_2 consumed/min/mg protein.

The protein concentration of the cytosolic fraction is evaluated by the method of Bradford [20].

Determination of enzymatic activity of superoxide dismutase (EC: 1.15.1.1)

The method adopted for dosage of the SOD is described by Beauchamp and Fridovich[21]. The assay is performed in a volume of 3ml consisting of 1.5ml of reactive medium (sodium cyanide 300µl of 20 µM, 300 µ l of nitroblue tetrazolium 45µM, 300 µl of 45 µM L-methionine, µl of 20 µM Riboflavin and 300 µl of phosphate buffer 50 µM), 1.2 ml of distilled water and 300 µl of the cytosolic fraction. To induce photoreaction of riboflavin and oxygen, the mixture is exposed to light from a 15 Watt lamp for 10 minutes. The reduction of Nitro Blue Tetrazolium by NBT superoxide anion formazan is followed by the spectro photometer at 560 nm, according to the formula:

$$\text{total inhibition} = \frac{\text{obtical density of blank} - \text{obtical density of sample}}{\text{optical density of blank}} \times 100$$

The enzymatic activity is the ratio of the total inhibition on the amount of protein in mg in the volume of the sample used calculated in terms of IU/mg protein as follows:

IU SOD / mg protein = (total inhibition) / (n × 50).

Statistical evaluation

The results obtained were statistically analyzed using the Student t test. All values are expressed as mean ± SEM Standards on average. The significance level was set at $p < 0.05$.

RESULTS

The results of the phytochemical tests carried out on the leaves of the three plants studied; indicate the richness of *Ajuga iva* leaves in total tannins, flavonoids, and coumarins. However, they are totally lacking anthocyanins, leuco-anthocyanins, irridoids, gallic tannins, alkaloids, senosides and mucilages. With regard to the leaves of *Salvia Officinalis*, the tests revealed a high richness in total tannins, gallic tannins, flavonoids, senosides and saponins. However, they are moderately rich in mucilages. On the other hand, they are devoid of coumarins, anthocyanins, leuco-anthocyanins, irridoids, alkaloids and quinones. Finally, the phytochemical screening of the leaf powder of *Peganum harmala* showed a high content of saponins, flavonoids, quinones, coumarins and alkaloids, and a medium mucilage content. On the other hand, they are totally lacking anthocyanins, leuco-anthocyanins, total tannins, irridoids, gallic tannins, senosides and quinones.

Extraction yield and dosage of polyphenols

The polyphenolic extract has a liquid appearance and a color ranging from yellowish brown to dark brown depending on the fraction. The yield obtained for 10 g of freeze-dried material is 5.6% for leaves of *Ajuga iva*, 4.5% for leaves of *Peganum harmala* and 1.43% for leaves of *Salvia officinalis*. From these results it can be seen that the best yield is obtained from the leaves of *Ajuga iva*. The total polyphenol contents obtained are between 127.7 ± 0.55 mg EAG / g (mixture) and 63.5 ± 0.2 mg EAG / g (powder for *Ajuga iva* leaves) (Table 1) .

Table 1: Quantitative evaluation of yields and concentrations of the various extracts

Extracts	yield (%)	Polyphenol content (mg EG A/ ml g)
Ajuga iva	5.6	61.5±0.12
Salvia officinalis	1.43	63.3±0.02
Peganum harmala	4.5	77.5±0.23
Mixture	-	127.7±0.55

Antioxidant Activity of polyphenols in the aerial part by the DPPH Method

The results obtained in the percentage inhibition test (PI %) of DPPH (2, 2-diphenyl-1-picrylhydrazyl) at the three plants are recorded in figure 1. We note that the phenolic extract of mixture has an IC50 of the order of 0.24 ± 0.0005 mg/ml stronger than the leaves of *Peganum harmala* (0.27 ± 0.002 mg/ml) and the leaves of *Salvia officinalis* (0.41 ± 0.0002 mg/ml). The capacities of the radical are classified in order: mixture> leaves of *Peganum harmala*> ascorbic acid> leaves of *Salvia officinalis*> leaves of *Ajuga iva*.

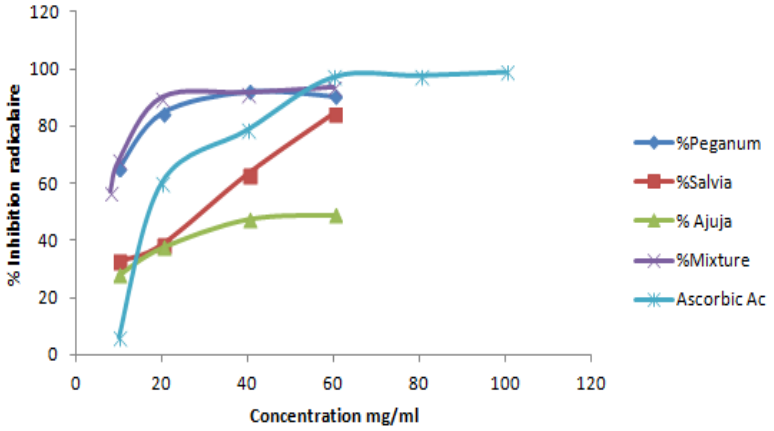

Figure 1. Antiradical power of the aqueous extract of the mixture

Evaluation of antioxidant activity in vivo

Effect of the aqueous extract of the mixture of Ajuga iva leaves, Salvia officinalis, Peganum harmala against diabetes induced by streptozotocin

The results obtained in our study showed that injection of streptozotocin induces diabetes characterized by severe loss of body weight in the group of diabetic control rats, after five days of treatment with saline solution. The recorded value is of the order of 14.94%, compared to the initial body weight. However, a highly significant improvement (P <0.001) in body weight in the group of diabetic rats treated with the aqueous extract of the mixture *of the* leaves of *Ajuga iva*, *Salvia officinalis*, *Peganum harmala* is recorded after five days, compared to the group of diabetic control rats.

For blood glucose, stability is observed in the blood level of glucose in the fifth day for the diabetic group treated with the aqueous extract at a daily dose of 300 mg/kg. The values recorded after pretreatment by the aqueous extract of the mixture was 1.07 ± 0.25

against an initial value (D_0) of 1.42 ± 0.03 g/l. On the other hand, a significant hyperglycemia ($p < 0.001$) was obtained from the unprotected rats against streptozotocin (1.87 ± 0.04 g/l) relative to the initial blood glucose of the same group (1.57 ± 0.03 g/l) (Tab.2).

Table 2: Variation of blood glucose and body weight in rats pretreated or not by the aqueous extract of the mixture

Groups and traitement	**weight (g)**		**Blood glucose (g/l)**	
rats	D_0	D +5	D_0	D+5
Healthy control rats	204±1.9	212±2.9	1.06±0.03	1.03±0.06
control + streptozotocin + NaCl	196±0.98	166.7±3.21	1.57±0.03	1.87±0.04
Treated + streptozotocin + *aqueous extract of the mixture*	147.75±1.1	174.16±6.59 **	1.42±0.03	1.07±0.31 *
Treated+ streptozotocin + Gallique Acid	94±2.04	103.5±2.2	1.29±0.03	1.09±0.14

*Values are given as mean ± standard deviation. Student test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: groups compared to the control group streptozotocin NaCl + 0.9%; + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$: group compared to him even at D_0 (before the injection of streptozotocin)*

Effect of the aqueous extract of the mixture on lipid peroxidation

The MDA concentrations were determined on liver cytosolic fractions. The variation of the level of malondialdéhyde (MDA) in liver cells is shown in Figure 2. According to our results, a significant increase ($p < 0.01$) of the MDA levels in untreated diabetic rats was observed (2.84 ±0.311nmol/g liver) compared to healthy control rats (0.0213±0.006/g liver). However, in diabetic rats pretreated with the aqueous extract of the mixture (0.83 ±0.030 nmol/g) and gallique acid (0.3±0.196 nmol/g, MDA levels decreased insignificantly ($p > 0.05$) compared with healthy control rats.

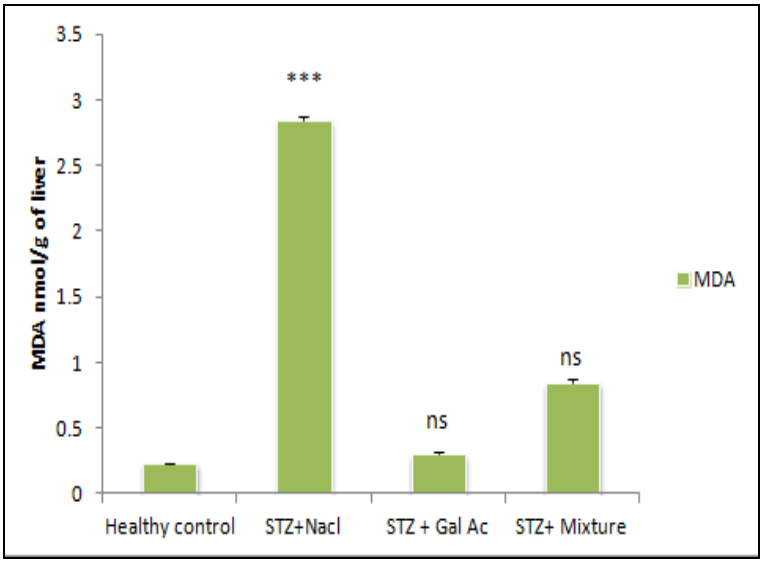

Figure2. Effect of aqueous extract of the mixture on the production of MDA in the liver cells

*The values are given on average ± Standard error. Student's test: * p <0.05 Groups compared to 0.9% NaCl control group.The values are given on average ± Standard deviation. Student's test: ns, * p <0.05, ** p <0.01: groups compared to the normal control group + NaCl.*

Effect of the aqueous extract of the mixture on the GSH in the liver

The variation of GSH level in liver cells in diabetic rats pretreated or not is shown in Figure 3. The results show that there is a significant decrease in GSH level in untreated diabetic rats with a value of 1,78 ± 0.48 nmol/g, compared to normal rats (5.23 ± 0.48 nmol/g liver). This decrease is probably due, partly to an increase in its use by the liver cells, and secondly, to a decrease in GSH synthesis , or an increase in its degradation during the oxidative stress caused by diabetes. However, we noticed a significant increase ($p < 0.05$) of GSH in the diabetic rats pretreated with the aqueous extract of the mixture and the gallic acid, respectively 4.18 ± 0.06 and 4.78 ± 0.867 nmol/g liver, relative to the GSH level of healthy control rats. Nevertheless, registered rates remain lower than that of healthy control rats.

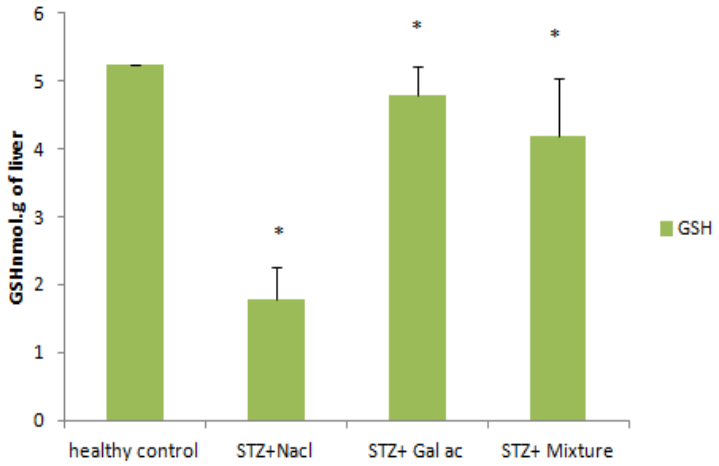

Figure3. Effect of the aqueous extract of the mixture on the cytosolic level of GSH in the liver

*The values are given on average ± Standard error. Student's test: * p <0.05 Groups compared to 0.9% NaCl control group.The values are given on average ± Standard deviation. Student's test: ns, * p <0.05, ** p <0.01: groups compared to the normal control group + NaCl.*

Effect of the aqueous extract of the mixture on catalase and superoxide dismutase SOD

The results of the effect of the aqueous extract of the mixture on the two enzymes are shown in Table 3. According to data carried by Table 3, the values of CAT activity in the pretreated rats with aqueous extract of the mixture (0.25 ± 0.05 UI/mg Pr) and Gallic acid (0.294 ± 0.5UI/mg Pr) approximate the healthy control rats (0.42 ± 0.3 IU/mg Pr). Furthermore, a significant decrease (p <0.01) of SOD activity in the liver in untreated diabetic rats compared to those recorded in control diabetes is noted.

Table 3: Effect of the aqueous extract of the mixture on the activity of antioxidant enzymes, CAT and SOD in streptozotocin induced rats and treated or not with the aqueous extract of the mixture

Groups of Rats	Protein mg/ml	Activity of CAT[UI/mg Pr]	Activity of SOD[UI/mg Pr]
Normal control+ saline solution	0.76±0.3	0.42±0.3	0.567±0.1
streptozotocin+ saline solution	0.83±0.5*	0.08±0.03 *	0.32±2.22*
Preteated streptozotocin +mixture	0.828±0.3 **	0.25±0.05 **	0.52±3.5**
Preteated streptozotocin +gallic acid	1.047±0.07 *	0.294±0.5*	0.64± 0.76 *

*The values are given on average ± Standard deviation. Student's test: ns, * p <0.05, ** p <0.01: groups compared to the normal control group + NaCl*

DISCUSSION

The phytochemical study of the three plants showed the existence of a real molecular biodiversity, conferring on the three plants important medicinal virtues which deserve to be valorized. Several authors have reported results similar to those obtained in this study. El-Hilaly [22] reported the presence of the same classes of chemical families found in leaves of *Ajuga iva*. Bruneton [23] reported the richese of *Salvia officinalis* in total tannins, gall tannins, flavonoids, senosides and saponins. Kartal [24] found in *Peganum harmala* the presence of coumarins, saponins, flavonoids, quinones and alkaloids. In general, the contents of polyphenol extracts vary not only from one plant to another but also according to the parameters of the polyphenol extraction, the particle size and the diffusion coefficient of the solvent. Solvent extraction at elevated temperature has been shown to yield higher yields of dry extracts than when obtained at room temperature and is higher for aqueous than methanol extract [25], which is consistent with our results. The phenolic content of a plant also depends on a number of intrinsic and extrinsic factors, particularly climatic conditions, maturity at harvest and storage conditions [26].

The DPPH test carried out on the various extracts revealed a significant free radical-inhibiting activity of the phenolic extract of the mixture relative to the extracts of the plants studied, an IC_{50} of 0.24 ± 0.0005 mg/ml. The anti-radical activity of the extracts is therefore relatively dependent on the total polyphenol content. The combination of the polyphenols of the three plants has the greatest inhibition of the DPPH radical. This result could be explained by a synergistic effect between the different biological constituents of the three plants. It is important to note that no antagonistic effect is observed for the prepared mixture. Being a mixture, this represents a potential for the development of synergistic and additive interactions. The synergism of antioxidants through exo-interactions has been given some attention. The combination of soy extracts, phytoestrogen alfalfa and cherry acerola extracts works synergistically to inhibit LDL oxidation in vitro [27] demonstrated that combinations of alpha-tocopherol and ascorbic acid with caffeic acid, catechin, epicatechin, myricetin, gallic acid, quercetin and rutin have more antioxidant activity. High that each of these compounds taken isolated for the oxidation of lipids.

Streptozotocin, by inducing experimental diabetes, causes excessive formation of reactive oxygen species that are highly toxic to cells, particularly cell membranes [28]. Thus, hyperglycemia caused excessive formation of reactive ROS oxygen species observed in untreated rats five days after streptozotocin injection. It is a key clinical manifestation of diabetes mellitus, and can increase the production of ROS, via many pathways including glucose oxidation, the polyol pathway, and protein glycation. On the other hand streptozotocin-treated rats pretreated with polyphenols have been shown to protect against the deleterious and diabetogenic effect of this toxin. This shows the high efficiency of infused all three plants in lowering blood sugar levels. In addition, in recent years, new bioactive hypoglycemic drugs isolated from plants have shown more effective antidiabetic activity. It is attributed to the richness of the aqueous extract of polyphenols known for their antidiabetic activities and whose mechanism could be a stimulation of the insulin secretion by the beta cells of the islets and/or the inhibition of the degradation process of the insulin[29].

Several mechanisms of antidiabetic action of polyphenols have been described. These metabolites inhibit glucose transport molecules in the intestines , decrease the expression of genes that control gluconeogenesis , increase glucose storage in the liver, and reduce glycogen degradation [30].

Indeed, the polyphenols improving the sensitivity of body cells to insulin, which allows reducing the incidence of type 2 diabetes[31]. In addition to the already mentioned mechanisms, these metabolites can inhibit hepatic glucose production by a purely energetic mechanism by modifying mitochondrial function, knowing that the glucose formation by the liver requires a significant energy input in the form of ATP. In this context,Viollet [32] showed that metformin reduces glucose production by reducing the production of ATP in liver cells. By this example, these authors suggest that another mechanism may also be involved in the antidiabetic action of metformin in a long term. It's about the activation of an enzyme sensitive to variations in energy levels in the cell (AMPK enzyme), which could reduce fat accumulation in the liver (hepatic steatosis) frequently associated with type 2 diabetes. This second way would trigger better insulin sensitivity and prevent hepatic glucose production.The present study revealed a significant increase in lipid peroxidation products (MDA), a decrease in the level of (GSH) activity of antioxidant enzymes SOD and CAT.

Lipid peroxidation is a process mediated by free radicals, leading to oxidative degradation of polyunsaturated lipids.The increase in MDA rate is the result of the increase of ROS that attack the polyunsaturated fatty acids of the cell membrane and causes lipid peroxidation [33]. The evaluation of lipid peroxidation produced in liver cells of unprotected rats is the result of a significant change that weakens the functioning of membranes by lower membrane fluidity and decreases the activity of enzymes and lees receptor membranes, probably due to the generation of free radicals by steptozotocin.

The results obtained suggest that the aqueous extract of the mixture was able to protect the liver tissues against oxidative stress and cytotoxic action induced by streptozotocin, improving the disease state of diabetes by inhibiting lipid.

Reduced glutathione (GSH) plays a multifaceted role in the antioxidant defense mechanism. It is a direct scavenger of free radicals, a cosubstrate necessary for GPx activity and is involved in the regeneration of oxidized vitamin E. Therefore, changes in the GSH redox state can be regarded as particularly sensitive indicator of oxidative stress [34].

The results suggest that the aqueous extract of the mixture could either increase glutathione biosynthesis or reduce oxidative stress leading to a decline in its degradation, or by influencing both mechanisms simultaneously.

It is thus clear that the enzymatic activity of the antioxidant system is kept at normal levels in rats pretreated with the aqueous extract of the mixture. The catalase (CAT) is a hemoprotein which catalyzes the reduction of hydrogen peroxide on H_2O and oxygen, and protects the tissues of hydroxyl radicals which are highly reactive [35]. The Superoxyde dismutase (SOD) is one of the most important antioxidant enzymes in the body's defense system. The major function of SOD is to catalyze the dismutation of the superoxide anion O_2^-. In H_2O_2 and therefore reduce toxic effects due to free radicals or other radicals which are derived from secondary reactions [36].

In diabetes, the decreased activity of antioxidant enzymes was reported in different tissues [34].The decrease in CAT activity could be the result of inactivation of the enzyme by the superoxide anion, by its glycation or by both mechanisms to gether. This also explains the inability of the SOD to protect the CAT.

Many studies have reported that the activity of CAT and SOD decreases in diabetes mellitus. Matkovics [37] found a decrease in SOD activity in all organs examined (liver, kidney, spleen, brain, heart, muscles, pancreas), except for lungs of rats made diabetic by alloxan or streptozotocin. The situation is compatible with our results where there was a significant decrease in SOD activity in the liver of alloxan induced rats compared with healthy rats group of witnesses.

The decrease in CAT activity may be the result of the inactivation of the enzyme by the superoxide anion, its glycation or both mechanisms at the same time. This also explains the SOD's inability to protect CAT. Modification of the equilibrium of antioxidant enzymes caused by decreased activity of SOD and CAT may be responsible for the lack of antioxidant defenses in the fight against ROS, which causes tissue damage [38].

In our work, the pretreatment of streptozotocin-treated rats with the aqueous extract of the mixture was able to maintain the antioxidant cellular defense systems (CAT and SOD) at their normal level. This balance is maintained under the effect of the antioxidant action of phenolic compounds against ROS generated by streptozotocin .

CONCLUSION

The treatment of rats with streptozotocin at 40 mg/kg produced diabetes characterized by hyperglycemia, a significant elevation of markers of oxidative stress and decreased enzymatic and non-enzymatic antioxidant defense system. The aqueous extract from the three plants caused a clear improvement in hepatic antioxidant status. In fact, the decrease of the concentration of MDA (5%), the increase of the reduced glutathione (GSH), the activity of superoxide dismutase (SOD) and catalase activity (CAT) on liver of diabetic rats treated with the extract clearly show the antioxidant properties of the aqueous extract of the mixture. Thus antidiabetic medicinal plants can provide a broad response to the complex problem of diabetes mellitus, and therapeutic perspectives for better care. Indeed they can play a role of food adjuvant as preventive or to increase the effectiveness of oral antidiabetic agent.

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